Supplemental Methods

General methods. Amino acid derivatives with the exception of N^{α}-(^{t}butoxycarbonyl)-thiazolidine which was purchased from Bachem (Torrance, CA), pre-loaded Wang resin, and coupling reagents were purchased from Novabiochem (San Diego, CA). *E. coli* BL21(DE3) and pLysS cells were purchased from Novagen (Madison, WI). ^{3}H-*S*-adenosyl methionine, was obtained from GE Healthcare (Piscataway, NJ). Restriction enzymes, T4 ligase, pTXB1 vector, and chitin resin were obtained from New England Biolabs (Ipswitch, MA). Criterion 15% and 4-20% Tris HCl, and Criterion 5% TBE gels were purchased from Biorad (Hercules, CA). Centricons were purchased from Sartorius (Goettingen, Germany). PCR purification and gel extraction kits were purchased from Qiagen (Valencia, CA). All other chemical reagents were purchased from Sigma-Aldrich (Milwaukee, WI) or Fisher Scientific (Pittsburgh, PA). Analytical and semi-preparative scale reverse-phase HPLC (RP-HPLC) were performed on a Hewlett-Packard 1100 series instrument using Vydac C18 columns (4 x 150 mm; 10 x 250 mm) at 1 and 4 mL/min, respectively. Unless otherwise noted, all analytical gradients were 0-73% B over 30 min (A: 0.1% trifluoroacetic acid (TFA) in water; B: 90% acetronitrile, 0.1% TFA in water). Preparative and process scale RP-HPLC were performed on a Waters DeltaPrep 4000 system connected to a Waters 486 tunable detector using Vydac C18 columns (22 x 250 mm; 50 x 250 mm) at 15 and 30 mL/min, respectively. Size-exclusion and ion-exchange chromatography were performed on an AKTA FPLC system from GE Healthcare equipped with a P-920 pump and a UPC-900 monitor. ESI-MS was performed on a SciexAPI-100 single quadrupole mass spectrometer. Linear peptide synthesis was performed on a Liberty synthesizer equipped with a Discovery microwave module (CEM,

Matthews, NC). Primer synthesis and DNA sequencing were performed by Integrated DNA Technologies and Genewiz, respectively.

Peptide synthesis. For peptide **1c**, the sequence corresponding to residues 117-125 of *Xenopus* H2B with an A117C substitution was synthesized on pre-loaded Wang resin using automatic solid-phase peptide synthesis with a 9-fluorenylmethoxycarbonyl (Fmoc) N^{α} protection strategy and using 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) for amino acid activation. Standard ^{t}butyl side-chain protection was used throughout with the following exceptions; the ε-amino group of K120 was protected with the 4-methyltrityl (Mtt) group, and the thiol group of C117 was installed as an N^{α}-(^{t}butoxycarbonyl)-thiazolidine (Boc-thiazolidine). The Mtt group on K120 was deprotected by successive incubations of the peptidyl-resin with 1% TFA in DCM containing 1% triisopropylsilane (TIS) for 10 min intervals, until no yellow color evolved. The sequence corresponding to residues 72-76 of ubiquitin were synthesized on the ε-NH_2 of K120 following Mtt deprotection. This ubiquitin branch was *N*-α-acetylated by incubation with acetic anhydride:DIEA:DMF (15:15:70) for 10 min. Following cleavage from the resin (as above), peptide **1c** was dissolved in 0.5 M aqueous methoxylamine, pH 5, for 3.5 h at room temperature to deprotect the N-terminal cysteine. Deprotected peptide **1c** was purified by RP-HPLC on a preparative scale using a 10-25% B gradient over 45 min. Peptide **1c** was characterized by ESI-MS [$(M+H)^{+}$ observed: 1,582.8 Da; expected: 1,582.8 Da] (Fig. S7).

Preparation of ubiquitin(1-75) and H2B(1-116)-α-thioesters. H2B(1-116)-α-MES, **5**, and HA-Smt3(2-97)-α-MES, **14**, were prepared as previously described (*1, 2*). Ubiquitin(1-75)-α-MES was prepared on large scale using a BioFlo 300 Benchtop

Fermenter operating in fermentation mode (New Brunswick Scientific, Edison, NJ). The fermenter was inoculated with *E. coli* BL21(DE3) transformed with pHub(1-75) (*2*). Cells were grown at 37 °C in Superbroth (MP Biomedicals) until an O.D. 600 of 21 was reached. Protein expression was induced with 0.5 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG) at 25 °C for 6 h under continuous supplementation with 50% glucose and 3x Superbroth and oxygen enrichment. pH was maintained at 7.0 with 5 N NaOH. After centrifugation at 6.8 kg for 15 min, the cell pellet was resuspended in 500 mL lysis buffer (50 mM Tris, 200 mM NaCl, 1 mM EDTA, 0.1 mM phenylmethylsulfonylfluoride (PMSF), 0.2 mg/mL deoxyribonuclease (DNase), pH 7.5) and lysed by passage through a French press. The insoluble material was removed by centrifugation at 26 kg for 30 min and the supernatant was incubated overnight at 4 °C with chitin resin (300 mL) pre-equilibrated in lysis buffer. The resin was washed with 2 L of wash buffer 1 (50 mM Tris, 200 mM NaCl, 1 mM EDTA, pH 7.2) and then incubated with cleavage buffer (50 mM Tris, 200 mM NaCl, 1 mM EDTA, 100 mM MESNa, pH 7.4) for 74 h, resulting in thiolysis of the intein fusion, forming ubiquitin(1-75)-α-MES, **2**. The column was eluted and the resin was washed with 200 mL cleavage buffer. The thiolysis reaction was repeated and the combined elution fractions further purified by process RP-HPLC using a 30-45% B gradient over 60 min, yielding 300 mg of lyophilized protein.

Ubiquitin(1-75)-α-MES with a L8A/I44A double mutant was prepared with the Quikchange Multi Site-directed Mutagenesis kit (Stratagene; La Jolla, CA) with the following primers: UbL8A (5'-GCAGATCTTCGTGAAGACTGCGACTGGTAAGACCATCACT-3') and UbI44A (5'-

CTGACCAGCAGAGGTTGGCCTTTGCTGGGAAACAGC-3') and pHub(1-75) (2) as a template, according to manufacturers instructions, generating pHub(1-75)L8A/I44A. *E. coli* BL21(DE3) cells transformed with pHub(1-75)L8A/I44A were grown in Luria-Bertani medium at 37 °C until mid-log phase, and protein expression was induced by the addition of 0.5 mM IPTG and allowed to continue at 25 °C for 8 h. After harvesting the cells, the cell pellet was resuspended in lysis buffer, lysed, and cleared as described above. Cleared lysates were incubated overnight at 4 °C with chitin resin (20 mL) pre-equilibrated in lysis buffer. The resin was washed with 100 mL of wash buffer 1 followed by 200 mL wash buffer 2 (50 mM Tris, 200 mM NaCl, 1 mM EDTA, pH 7.4). The resin was then incubated with 20 mL cleavage buffer (50 mM Tris, 200 mM NaCl, 1 mM EDTA, 100 mM MESNa, pH 7.4) for 48 h, resulting in thiolysis of the intein fusion, forming ubiquitin(1-75)L8A/I44A-α-MES, **15**. The column was eluted and the resin was washed with 20 ml cleavage buffer. The thiolysis reaction was repeated twice and the combined elution fractions further purified by preparative RP-HPLC using a 25-55% B gradient over 45 min. The identity of the purified protein **15** was verified by ESI-MS [$(M+H)^+$ observed: 8,548 ± 1 Da; expected: 8,548 Da] (Fig. S3). The identities of proteins **2**, **5**, and **14** were also verified by ESI-MS (Fig. S3).

Semisynthesis of tr-uH2B. Peptide **1c** (0.6 mg, 0.38 μmol), and H2B(1-116)-MES, **5** (2.1 mg, 0.16 μmol), were dissolved in ligation buffer to a final volume of 160 μL. The pH of the resulting solution was increased to 7.8 with 5 N NaOH and the reaction was allowed to proceed for 19 h prior to adding fresh TCEP to a final concentration of 25 mM. The ligation product, **17**, was purified using semi-preparative RP-HPLC with a 42-52% B gradient over 45 min, yielding 1.1 mg of protein. Protein **17** (1.1 mg, 76 nmol)

was desulfurized using Raney nickel as described above and purified using semi-preparative RP-HPLC with a 42-52% B gradient over 45 min, yielding 0.3 mg of tr-uH2B, **18**. Protein **18** was characterized by ESI-MS. [$(M+H)^+$ observed = 14,400 ± 3 Da; $(M+H)^+$ expected = 14,399 Da]

Semisynthesis of sH2B(cys). To generate sH2B(cys), HA-Smt3(2-97)-α-MES, **14**, was substituted for ubiquitin(1-75)-α-MES in the first step of the synthesis. Peptide **1b**, (5.7 mg, 5.1 μmol) and protein **14**, (8.4 mg, 0.68 μmol) were combined in 338 μL of ligation buffer. The pH was adjusted to 7.8 using 5 N NaOH and the reaction was allowed to proceed for 4 h at room temperature, forming ligation product **19**. To this solution was added 338 μL 50% HPLC buffer B and 169 μL 4 M methoxylamine and the pH was adjusted to 5. After 12 h at room temperature the deprotected protein, **20**, was purified by semipreparative RP-HPLC using a 35-60% B gradient, yielding 8.1 mg of product. Protein **20** (6.8 mg, 0.51 μmol) and protein **5** (5.6 mg, 0.43 μmol) were combined in 254 μL of ligation buffer. After 52 h at room temperature, fresh TCEP was added to a final concentration of 20 mM and the ligation product was purified by semipreparative RP-HPLC using a 42-52% B gradient over 45 min, yielding 5.5 mg sH2B(cys), **21** [$(M+H)^+$ observed = 26,228 ± 9 Da; $(M+H)^+$ expected = 26,224 Da]. No protein was recovered following Raney nickel desulfurization of protein **21** using procedures detailed above.

Semisynthesis of mut-uH2B. To generate mut-uH2B, ubiqutin(1-75)L8A/I44A-α-MES, **15**, was substituted for ubiquitin(1-75)-α-MES in the first step of the synthesis. Peptide **1b**, (0.6 mg, 0.54 μmol) and protein **15**, (5.0 mg, 0.58 μmol) were combined in 160 μL of ligation buffer. The pH was adjusted to 7.8 using 5 N NaOH and the reaction was allowed to proceed for 2 h at room temperature, forming ligation product **22**. To this

solution was added 160 μL 50% HPLC buffer B and 46 μL 4 M methoxylamine and the pH was adjusted to 5. After 12 h at room temperature the deprotected protein, **23**, was purified by semipreparative RP-HPLC using a 32-42% B gradient, yielding 2.4 mg of product. Protein **23** (0.8 mg, 80 nmol) and protein **5** (1.2 mg, 90 nmol) were combined in 50 μL of ligation buffer. After 41 h at room temperature, fresh TCEP was added to a final concentration of 20 mM and the ligation product was purified by semipreparative RP-HPLC using a 42-52% B gradient over 45 min, yielding 0.2 mg protein **24.** Radical initiated desulfurization was performed on protein **24** (200 μg, 9.0 nmol) as described above using 1/5th the total reaction volume and the product was purified by analytical RP-HPLC using a 0-73% B gradient over 30 min, yielding 150 μg mut-uH2B, **25** [$(M+H)^+$ observed = 22,293 ± 7 Da; $(M+H)^+$ expected = 22,295 Da]

Ubiquitin hydrolysis reaction. UCH-L3 (Boston Biochem, Cambridge, MA) was diluted to a concentration of 5 μM in reduction buffer (50 mM Tris HCl, 150 mM NaCl, 15 mM DTT, pH 7.5) and incubated at room temperature for 15 min. Reduced UCH-L3 (1 μM) was then combined with either uH2B or uH2BG76A (10 μM), respectively, in 25 μL hydrolysis buffer (50 mM Tris, 150 mM NaCl, 1 mM DTT, pH 7.5) and transferred to a 37 °C water bath. After 2 and 10 min, 5 μL of each of the samples was removed for analysis by SDS-PAGE. At 10 min, the concentration of UCH-L3 was doubled and a final 5 μL sample was removed after 30 min. Controls were performed without histone and without UCH-L3. Samples were separated on a Criterion 15% Tris HCl gel and stained with Coomassie. An equivalent reaction was performed on 2.5x scale and submitted to LC-MS analysis using a QStar QQTOF LC-MS (Applied Biosystems) to verify hydrolysis of the isopeptide bond (Fig. S5).

Western blotting. 250 and 125 ng of uH2B and uH2BG76A were separated on a Criterion 15% Tris HCl gel and transferred to a PVDF membrane using previously described transfer conditions (3). A Western blot was performed using a linkage specific α-uH2B antibody (cat. # MM-0029, Medimabs, Quebec, Canada) at 1:3,000. The blot was stripped and probed against an α-ubiquitin antibody (cat. # MAB701, R & D Systems) at 1:1,000. The blot was then stained with Ponceau.

Recombinant histone preparation. Recombinantly expressed *Xenopus* histones H2A, H2B, H3, H4, and H3K79R were prepared as previously described (1) with an additional purification by process RP-HPLC using a 40-60% B gradient, over 60 min. H2AE64A, H2AN68A, H4R17/19A mutations were performed using a Quikchange XL II kit (Stratagene) according to the manufacturer's instructions, using the following primers: H2AE64A-forward (5'-CTGACCGCTGAGATTTTGGCATTGGCCGGGAATG-3'), H2AE64A-reverse (5'-CATTCCCGGCCAATGCCAAAATCTCAGCGGTCAG-3'), H2AN68A-forward (5'-TTTGGAATTGGCCGGGGCTGCGGCCCGTGATAAC-3'), H2AN68A-reverse (5'-GTTATCACGGGCCGCAGCCCCGGCCAATTCCAAA-3'), H4R17/19A-forward (5'-CTGGGTAAAGGTGGTGCTAAAGCTCACGCTAAAGTTCTGCGTGACAACA-3') and H4R17/19A-reverse (5'-TGTTGTCACGCAGAACTTTAGCGTGAGCTTTAGCACCACCTTTACCCAG-3'). The resultant plasmids were transformed *E. coli* BL21(DE3), and the mutant proteins expressed and purified as described above.

Preparation of DNA for nucleosome formation. For small-scale nucleosome reconstitutions, 601-147-1 was prepared by PCR as previously described (1). For large-

scale nucleosome reconstitutions, the palindromic α-satellite sequence was prepared similarly to previously reported (4). Briefly, half of the α-satellite palindromic sequence was amplified by PCR using template DNA sequences α-sat-temp-forward (5'-ATCAATATCCACCTGCAGATTCTACCAAAAGTGTATTTGGAAACTGCTCCATCAAAAGGCATGTTCAGCGGAATTC-3') and α-sat-temp-reverse (5'-GAATTCCGCTGAACATGCCTTTTGATGGAGCAGTTTCCAAATACACTTTTGGTAGAATCTGCAGGTGGATATTGAT-3') and primers α-sat-forward (5'-CGGGATCCGATATCAATATCCACCTGCAG-3') and α-sat-reverse (5'-AAGGAAAAAAGCGGCCGCAGATCTGAATTCCGCTGAACATGCCTTTTG-3'). The PCR product was digested with BamHI and NotI and ligated into a similarly digested pCDNA3 vector. The resultant vector was digested with BamHI and NotI, allowing the α-satellite sequence to be isolated. The vector was also digested with BglII and NotI, allowing the pCDNA3 vector containing one copy of the α-satellite sequence to be isolated. These digestion products were ligated together, generating a vector with two copies of the α-satellite sequence, while obliterating the internal restriction site. This process was repeated until a vector containing 64 copies of the α-satellite sequence, pαSat64 was generated.

E. coli DH5a cells (Invitrogen, Carlsbad, CA) transformed with pαSat64 were amplified in the fermenter as described above for 15 h at 37 °C. Harvested cells were purified using a Plasmid Giga kit (Qiagen) according to manufacturer's instructions. Purified pαSat64 was digested with EcoRI and EcoRV to excise the α-satellite sequence. The vector was removed by precipitation with 7.5% PEG-6000. The α-satellite sequence was ethanol precipitated and further purified away from the small fragments of DNA

between the EcoRI and EcoRV restriction sites by gel filtration using a G50 sepharose (GE Healthcare). This sequence was self ligated with T4 ligase and then digested once more with EcoRV to generate the mature 146 bp α-satellite palindromic sequence and subsequently purified by chloroform extraction and ethanol precipitation.

Nucleosome formation. Histone octamer formations and small-scale mononucleosome reconstitutions were preformed by dilution as previously described using 601-147-1 (1). These mononucleosomes were used for all experiments except kinetics experiments. For kinetics experiments, large-scale nucleosome reconstitution was performed with the 146 bp α-satelite palindromic DNA sequence following procedures similar to those previously reported (4). Briefly, octamers were mixed with α-satellite DNA in ratios optimized using the small-scale reconstitution protocol described above, at 5 μM DNA concentration, and the NaCl concentration was adjusted to 2 M. The resultant solution was dialyzed against 400 mL start buffer (1.4 M KCl, 10 mM Tris HCl, 0.1 mM EDTA, 1 mM DTT, pH 7.5) at 4 °C for 70 min. The dialysate was diluted with end buffer (10 mM KCl, 10 mM Tris HCl, 0.1 mM EDTA, 1 mM DTT) to reach a final KCl concentration of 1.2 M and dialysis was continued for 70 min. Subsequent dialysis steps of at least 2 h with 1.0 M KCl, 70 min in 0.8 M KCl, and 70 min in 0.6M KCl, were followed by two dialysis steps of at least 3 h each in end buffer. Nucleosomes were concentrated to approximately 200 μL in Vivaspin 2 centricons and purified using a Model 491 Prep Cell from Biorad as previously described (4). Fractions deemed pure by native gel were combined and concentrated to 30 μM using Vivaspin 500 centricons. Aliquots were stored in 20% glycerol at 15 μM concentrations at -80 °C.

Supplemental References

(1) McGinty, R. K., Kim, J., Chatterjee, C., Roeder, R. G., and Muir, T. W. (2008) Chemically ubiquitylated histone H2B stimulates hDot1L-mediated intranucleosomal methylation. *Nature 453*, 812-6.

(2) Chatterjee, C., McGinty, R. K., Pellois, J. P., and Muir, T. W. (2007) Auxiliary-mediated site-specific peptide ubiquitylation. *Angew Chem Int Ed Engl 46*, 2814-8.

(3) Chiang, K. P., Jensen, M. S., McGinty, R. K., and Muir, T. W. (2009) A Semisynthetic Strategy to Generate Phosphorylated and Acetylated Histone H2B. *Chembiochem*.

(4) Dyer, P. N., Edayathumangalam, R. S., White, C. L., Bao, Y., Chakravarthy, S., Muthurajan, U. M., and Luger, K. (2004) Reconstitution of nucleosome core particles from recombinant histones and DNA. *Methods Enzymol 375*, 23-44.

Supplemental Figure Legends

Figure S1. Semisynthesis of uH2B. a, Synthetic scheme for uH2B synthesis. v) EPL was used to ligate peptide **1a** to protein **2**, forming branched protein **8**. vi) Ligation product **8** was irradiated at 365 nm, affording deprotected product **9**. vii) Ligation of protein **9** to protein **5**, forming uH2BA117C, **10**. viii) Raney nickel or radical-initiated desulfurization of protein **10**, forming uH2B, **11**. **b,** RP-HPLC chromatogram of uH2B, **11**. **c,** ESI-MS spectrum of purified uH2B, **11**. Charge states are labeled. [$(M+H)^+$ observed = 22,366 ± 4 Da (s.d.). $(M+H)^+$ expected = 22,365 Da.]

Figure S2. Synthesis of H2B-C peptide, 1b. a, Synthetic scheme for peptide **1b** preparation. Residues 117-125 of H2B were synthesized on the solid support, replacing A117 with a thiazolidine, affording peptidyl resin **12**. ix) Orthogonal deprotection of K120, followed by coupling of cysteine through an isopeptide bond, giving peptidyl resin **13.** x) Simultaneous side-chain deprotection and cleavage from the solid support, yielding peptide **1b**. **b,** RP-HPLC of purified peptide **1b**. **c,** ESI-MS spectrum of peptide **1b**.

Charge states are labeled. $(M+H)^+$ observed = 1,115.9 Da. [$(M+H)^+$ expected = 1,115.3 Da.] An asterisk represents MS fragmentation of peptide.

Figure S3. Characterization of protein-α-thioesters used in EPL reactions. RP-HPLC chromatograms (top panels) and ESI-MS spectra (bottom panels) for the following protein-α-thioesters: **a,** ubiqutin(1-75)-α-MES, **2** [$(M+H)^+$ observed = 8,632 ± 2 Da (s.d.). $(M+H)^+$ expected = 8,632 Da.]; **b,** H2B(1-116)-α-MES, **5** [$(M+H)^+$ observed = 12,991 ± 3 Da. $(M+H)^+$ expected = 12,991 Da]; **c,** HA-Smt3(2-97)-α-MES, **14** [$(M+H)^+$ observed = 12,414 ± 3 Da. $(M+H)^+$ expected = 12,414 Da.]; and **d,** ubiquitin(1-75)L8A/I44A-α-MES, **15** [$(M+H)^+$ observed = 8,548 ± 1 Da. $(M+H)^+$ expected = 8,548 Da.]. Charge states are labeled. MES = 2-mercaptoethane sulfonic acid.

Figure S4. Characterization of intermediate products in the semisynthesis of uH2BG76A. RP-HPLC chromatograms (top panels) and ESI-MS spectra (bottom panels) of the following intermediate products: **a,** ligation product, **3** [$(M+H)^+$ observed = 9,609 ± 2 Da (s.d.). $(M+H)^+$ expected = 9,606 Da.]; **b,** deprotected ligation product, **4** [$(M+H)^+$ observed = 9,593 ± 2 Da. $(M+H)^+$ expected = 9,594 Da]; and **c,** ligation product, **6** [$(M+H)^+$ observed = 22,444 ± 5 Da. $(M+H)^+$ expected = 22,443 Da.]. Charge states are labeled. Crude RP-HPLC spectrum is shown for protein in **a**.

Figure S5. Verification of isopeptide bond hydrolysis. a, LC-MS spectrum of ubiquitin hydrolyzed from uH2B by UCH-L3 (top panel) and resultant deconvoluted mass (bottom panel). **b,** LC-MS spectrum of ubiquitin G76A hydrolyzed from uH2BG76A by UCH-L3 (top panel) and resultant deconvoluted mass (bottom panel). Charge states are labeled.

Figure S6. Characterization of unmodified, wild-type and mutant *Xenopus leavis* histones. ESI-MS spectra of the following histones: **a,** H2A [$(M+H)^+$ observed = 13,949 ± 1 Da (s.d.). $(M+H)^+$ expected = 13,951 Da.]; **b,** H2B [$(M+H)^+$ observed = 13,814 ± 3 Da. $(M+H)^+$ expected = 13,818 Da]; **c,** H3 [$(M+H)^+$ observed = 15,270 ± 1 Da. $(M+H)^+$ expected = 15,272 Da.]; **d,** H4 [$(M+H)^+$ observed = 11,236 ± 1 Da. $(M+H)^+$ expected = 11,237 Da.]; **e,** H2AE64A [$(M+H)^+$ observed = 13,894 ± 3 Da. $(M+H)^+$ expected = 13,893 Da.]; **f,** H2AN68A [$(M+H)^+$ observed = 13,907 ± 3 Da. $(M+H)^+$ expected = 13,908 Da.]; **g,** H4R17/19A [$(M+H)^+$ observed = 11,067 ± 3 Da. $(M+H)^+$ expected = 11,067 Da.]; and **h,** H3K79R [$(M+H)^+$ observed = 15,300 ± 3 Da. $(M+H)^+$ expected = 15,300 Da.]. Charge states are labeled.

Figure S7. Synthesis of tr-uH2B-C peptide, 1c. a, Synthetic scheme for peptide **1c** preparation. Residues 117-125 of H2B were synthesized on the solid support, replacing A117 with a thiazolidine, affording peptidyl resin **12**. xi) Orthogonal deprotection of K120, followed by coupling of *N*-α-acetylated ubiquitin(72-75) through an isopeptide bond, giving peptidyl resin **16.** xii) Simultaneous side-chain deprotection and cleavage from the solid support, followed by cysteine deprotection with methoxylamine at pH 5, yielding peptide **1c**. **b,** ESI-MS spectrum of peptide **1c**. Charge states are labelled. [$(M+H)^+$ observed = 1,582.8 Da. $(M+H)^+$ expected = 1,582.8 Da.]

Figure S8. Characterization of semisynthetic proteins for structure activity relationship analysis. a, Schematic of tr-uH2B semisynthesis. xiii) EPL was used to ligate peptide **1c** to H2B(1-116)-α-thioester, **5**, to give tr-uH2B A117C, **17**. xiv) Desulfurization of protein **17**, yielding tr-uH2B, **18**. **b,** ESI-MS spectra of isolated tr-uH2B, **18** [$(M+H)^+$ observed = 14,400 ± 3 Da (s.d.). $(M+H)^+$ expected = 14,399 Da.]. **c,** Schematic of sH2B(cys) semisynthesis. xv) EPL was used to ligate peptide **1b** to HA-

Smt3(2-98)-α-thioester, **14**, to give protein **19**. xvi) Ligation product **19** was treated with methoxylamine at pH 5, affording protein **20**. xvii) Ligation of protein **20** to protein **5**, forming sH2BA117C/G98C (sH2B(cys)), **21**. **d,** ESI-MS spectra of isolated sH2B(cys), **21** [$(M+H)^+$ observed = 26,228 ± 9 Da $(M+H)^+$ expected = 26,224 Da.]. **e,** Schematic of mut-uH2B semisynthesis. xviii) EPL was used to ligate peptide **1b** to ubiquitin(1-75)L8A/I44A-α-MES, **15**, to give protein **22**. xix) Ligation product **22** was treated with methoxylamine at pH 5, affording protein **23**. xx) Ligation of protein **23** to protein **5**, forming protein **24**. xxi) Desulfurization of protein 24 to give mut-uH2B, **25**. **f,** ESI-MS spectra of mut-uH2B, **25** [$(M+H)^+$ observed = 22,293 ± 7 Da $(M+H)^+$ expected = 22,295 Da.].

Figure S9. Dot1L assay with exogenous ubiquitin. Dot1L assay with ^{3}H SAM and unmodified nucleosomes with and without 1 mM exogenous ubiquitin. Assays samples were separated by native gel electrophoresis and visualized with ethidium bromide staining (top panel). Quanitification of ^{3}H methyl incorporation was performed by filter-binding followed by liquid scintillation counting (bottom panel). Error bars represent one s. d. (n = 4).